## REMARKS

Applicants acknowledge that the Examiner has found the subject matter of claims 4-8, 17-18, 21, 25, 26, 28, 29, 31, and 33 free from prior art and allowable. Claims 35-40 have been withdrawn. Upon entry of this amendment, claims 1-34 and 41 are presently pending.

Claim 1 has been amended to more distinctly point out the characteristics and features of the claimed subject matter. More particularly, claim 1 has been amended to clarify that the modified peptide is a "post-translationally" modified peptide.

Claims 3, 18, and 32 have been amended, as suggested by the Examiner, to recite the full names of certain abbreviations recited in the claims.

Claims 15, 17, 30, and 31 have been voluntarily amended to more distinctly point out the characteristics and features of the claimed subject matter. More particularly, claim 15 has been amended to clarify that the antibody "specifically binds" the target motif. Claim 17 has been amended to clarify that the antibody binds a motif that "consists of" rather than comprises the recited preferred motifs. Lastly, claims 30-31 have been amended to correct a typographic error by replacing "modified peptide" with "phosphopeptide."

New dependent claim 41 has been added, and is drawn to certain preferred species of post-translational modification within the scope of the invention, namely "acetylation, glycoslyation, or methylation."

These amendments are supported throughout the specification as originally filed, for example, in the Background (pgs. 1-6 generally); p. 7, lines 10-16; p. 21, lines 7-15; p. 34, lines 5-15. The present amendments do not introduce new matter.

### RESTRICTION REQUIREMENT

The Examiner has required Applicants to reaffirm their provisional election (made by Applicants' attorney via telephone on April 6, 2005) to prosecute the subject matter of Group I (claims 1-34) in the present application. Applicants hereby reaffirm their election of Group I, and reserve the right to pursue non-elected subject matter (of Groups II and III) in a later divisional or continuation filing.

## §112, 2ND PARAGRAPH, INDEFINITENESS REJECTIONS

The Examiner has rejected claims 1-34 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. The Examiner argues that the term "modified peptide" in claim 1 renders the claim indefinite because the nature of the modification is unclear, and that the recitation of abbreviations (without corresponding full names) in claims 3, 18, 30, and 32, renders those claims indefinite. The Examiner also argues that claim 7 is vague because it is not clear if the recited enzyme is immobilized to the same support as the antibody.

Without necessarily agreeing with the Examiner, in order to expedite prosecution and allowance of this case, Applicants have amended claim 1 to clarify that the modified peptide is a "post-translationally" modified peptide, as described throughout the specification. Applicants have also amended claims 3, 18, and 30 to recite the full names corresponding to the recited abbreviations (e.g. LC-MS means liquid-chromatography-MS, etc.; AKT, however, has no full alternative name). Accordingly, these claims are clear and definite, and Applicants respectfully request that these rejections be withdrawn.

Applicants submit that pending claim 7, as filed, is clear and definite to those of skill in the art to which the invention pertains, in view of the teachings of the specification. Dependent claim 7 is drawn to a preferred embodiment of the method in which an immobilized enzyme (rather than a soluble enzyme) is employed to prepare a digested biological sample (which is then contacted with the immobilized antibody according to claim 1). Claim 7 depends from claim 6, which itself depends from claim 5, which itself depends from claim 1. Read together, these dependent claims make clear that the proteinaceous preparation (in this preferred embodiment of claim 7) to be contacted with the immobilized antibody of step (b) in claim 1 is a biological sample that has first been digested with an immobilized proteolytic enzyme. It is clear to those of skill in the art that the immobilization referred to in claim 7 is independent of the immobilization of the antibody referred to in step (b) of claim 1, since, according to claim 7, one must have the digested biological sample in hand before it can be contacted with the immobilized antibody as recited in step (b). Accordingly, claim 7 is definite and clear, and Applicants respectfully request that this rejection be withdrawn.

## DOUBLE-PATENTING (STATUTORY) REJECTIONS

The Examiner has provisionally rejected claims 1-29 under 35 U.S.C. §101 for "statutory" double patenting, as allegedly claiming the same invention as that of claims 1-29 of co-pending application USSN 10/175,486 (Rush *et al.* -- also owned BY CELL SIGNALING TECHNOLOGY, INC., the assignee of the present application).

Since the rejection is provisional, Applicants respectfully request that this rejection be held in abeyance until such time as the present application or cited co-pending application issues as a patent, at which time Applicants will cancel or amend any identical claims in the remaining application.

**§102(E) NOVELTY REJECTIONS**

The Examiner has rejected claims 1-3, 9-12, 19, 20, 22-24, and 27 under 35 U.S.C. §102(e) as allegedly being anticipated by Little *et al.* (U.S. Pat. No. 6,322,970, "Mass Spectrometric Detection of Polypeptides" (issued Nov. 27, 2001) (hereinafter "Little")). The Examiner asserts that Little discloses a method for isolating a "modified peptide" using an immobilized "modification-specific antibody" within the scope of the present claims. Applicants respectfully disagree, and submit that the Examiner overextends the teachings of Little, which does not describe, nor is enabled for, the presently claimed subject matter.

Little discloses a method for isolating a target peptide using an immobilized capture molecule. As the Examiner correctly notes, Little discloses that a target polypeptide can be isolated from a biological sample using an immobilized target-specific antibody that can capture the target polypeptide. However, Little does not, in any way, disclose that an antibody specific for a single post-translationally modified amino acid or motif can be successfully employed to selectively isolate a population of post-translationally modified peptides (containing the modified residue or motif). Rather, Little specifically describes using either an antibody that is specific for the target peptide sequence itself, or a tag-specific antibody (such as a His-tag antibody) that specifically binds a single target peptide or protein labeled with such artificial tag. (*See* Little, *e.g.*, at column 4, lines 24-37; column 15, lines 15-45; column 20, lines 21-24).

Indeed, in Example 1, which is the *only* example of the disclosed method provided in Little, the well-known His-tag system was used to selectively isolate an amplified target peptide fused to His-tag using an immobilized His-tag-specific antibody. The His-tag is an artificial label introduced to proteins for purification purposes, and is not a naturally occurring post-translational modification. His-tag is not a modification within the scope of the present invention, and the His-tag antibody (as with all tag-specific antibodies) is not a modification-specific antibody (*i.e.* one that binds at least one modified amino acid either alone or as part of a motif) within the meaning and scope of the present invention (see Specification, p. 21, line 27 onward).

Thus, Little fails to describe, possess, or enable a method for selectively and directly isolating, from a complex mixture of peptides, a population of post-translationally modified peptides (containing a common modified amino acid or motif) using an immobilized antibody that specifically binds the post-translationally modified amino acid (either alone or as part of a motif). Indeed, as described in the Background of the present application, the need solved by the invention is a simple,

reproducible method for simply and directly isolating and identifying desired post-translationally modified peptides from a complex peptide mixture, *without the need* for multiple, time-consuming preparative steps that limited prior art approaches. In contrast, Little expressly discloses the desirability of "conditioning a polypeptide" before its isolation by using a variety of different peptide modification, additions, and derivations to improve the mass spectrum resolution of the target peptide. Likewise, in the sole example provided in Little, the target peptide is first produced and amplified as an artificially-tagged fusion protein using the well-known Qiagen QIAEXPRESS™ protein purification system (*see* Little, Example 1). This type of complicated manipulation prior to isolation distinguishes the method disclosed in Little from that of the present invention, which does not require them. The distinct advantages of the instant invention over prior approaches like Little are discussed at length in the Specification (*see* pgs. 20-21).

The novelty and inventiveness of the presently claimed subject matter over prior art approaches, like that of Little, has been evidenced by several publications in the field of peptide isolation and identification by mass spectrometry. For example, Mann *et al., Trends in Biotech. 20:* 261-268 (2002) (cited and discussed in the Background (Ref. CG)), is a review of peptide isolation and phosphoproteomic mass spectrometry approaches authored by a recognized leader in the field. This review expressly states the prevailing view (at the time the present application was filed) that phospho-specific antibodies were *not* suitable for isolating phosphorylated peptides from mixtures, due to various technical limitations. Thus, the present invention was in fact a novel and surprising advance over prior approaches, since the prior art taught away from the suitability of modification-specific antibodies to isolate post-translationally modified peptides. The novelty of the claimed subject matter is further underscored by a recent review article of the method (which Applicant had earlier published in a scientific journal after the filing of this application) that appeared in *Nature Biotechnology* (*see* Conrads *et al., Nat. Biotech. 23:* 36-37 (Jan. 2005), attached (Ref. CZ, presently submitted). In the review, the authors expressly conclude that the inventors "address the deficiency" in prior art proteomics approaches for isolating phosphopeptides, and highlight several of the problems (*e.g.* low abundance of phosphopeptides from complex mixtures, need for enrichment, etc.) with prior art approaches that are simply and powerfully solved by Applicants' method.

Accordingly, the subject matter of claims 1-3, 9-12, 19, 20, 22-24, and 27 is novel and inventive over Little (as are the other claims found allowable), and Applicants respectfully request that the rejection of these claims be withdrawn.

**§103(A) OBVIOUSNESS REJECTIONS**

**(I)** The Examiner has rejected claim 12 under 35 U.S.C. §103(a) as allegedly being

obvious given Little (U.S. Pat. No. 6,322,970; *see supra.*) in view of Pidgeon (U.S. Pat. No. 6,579,720, "Method for Activity Profiling Compound Mixtures," (issued June 17, 2003) (hereinafter "Pidgeon")). The Examiner acknowledges that Little fails to teach the use of an immunoaffinity column coupled to a mass spectrometer (MS), but alleges that Pidgeon discloses such an element, thereby rending the presently claimed subject matter obvious. Applicants respectfully disagree, and submit that the Examiner has failed to establish a *prima facie* showing that the method of claim 12 is obvious in view of the cited references.

It is axiomatic that an Examiner must establish *a prima facie* case of obviousness by establishing three elements: (i) that there is some suggestion or motivation in the references themselves – or if not, then in the knowledge generally available to those of skill in the art – to combine the teachings of the references; (ii) that there is some reasonable expectation of success, as evidenced by the cited references and/or other prior art, in so combining the teachings, and (iii) that the cited references teach or suggest each and every limitation of the claimed subject matter. *See* MPEP §§2142, 2143, *citing, e.g. In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991). The mere fact that references can be combined is *not* sufficient to establish desirability or motivation to do so (*see* MPEP §2143.01, citing *In re Mills*, 916 F.2d 680) (Fed. Cir. 1990)). In the present case, a *prima facie* showing of obviousness has not been established because none of the three required elements has been met.

The first required prong of a *prima facie* showing has not met because there is no suggestion or motivation provided in Little or Pidgeon, or the art to which each relates, to combine the teachings of the references. Little relates to the field of peptide and protein isolation and characterization using mass spectrometry (MS). In contrast, Pidgeon relates to the field of chemical compound screening using MS. Pidgeon discloses the use of high performance liquid chromatography (HPLC) to separate and resolve chemical compounds, such as pharmaceuticals, in a mixture prior to their analysis on a mass spectrometer. The chemical compounds are resolved in the HPLC column by *chemical* parameters such as polarity, charge, molecular mass, weight, etc. (*see* Pidgeon, *e.g.* at column 133, lines 20-30). Little, on the other hand, discloses the use of immobilized capture molecules (such as artificial tag-specific antibodies) to affinity isolate a target *peptide* prior to MS characterization. There is no motivation or suggestion in the references themselves or in the general knowledge in the field to which each relates to combine the teachings of Little and Pidgeon since, in fact, these references are in different fields and pertain to different isolation technology. Accordingly, the first required prong of a *prima facie* showing has not been met.

Indeed, as discussed earlier, Little itself fails to disclose or suggest the presently claimed method for selectively and directly isolating a population of post-translationally modified peptides from a complex mixture of peptides using an immobilized antibody that specifically binds a post-

translationally modified amino acid (alone or as part of a motif). The limited teachings of Pidgeon regarding use of HPLC coupled with MS to screen chemical compounds fail to cure the deficiencies of Little. Thus, the references, taken alone or together, fail to suggest or make obvious the presently claimed subject matter.

The second required prong of a *prima facie* showing of obviousness has also not been met, since there is no evidence in the cited references themselves, (or in the art of peptide isolation generally) to indicate that a skilled artisan would have a reasonable expectation of success, *in arriving at the presently claimed invention*, were they to combine the teachings of Pidgeon with those of Little. Little itself, as previously discussed and acknowledged by the Examiner, fails to disclose or suggest the method of claim 12. An artisan familiar with the peptide isolation approach disclosed in Little would not have a reasonable expectation of success in utilizing the HPLC techniques disclosed in Pidgeon since the latter are directed to isolating *chemical* compounds by chemical parameters, not peptides by immunoaffinity, and are thus not suitable for the latter. However, even if such artisan were, *arguendo*, to have a reasonable expectation of success in combining the two teachings, the expectation of success would only extend to performing the peptide isolation method disclosed in Little and incorporating an HPLC fractionation. Such artisan would have no expectation of success of being able to directly immunoaffinity isolate a desired population of post-translationally modified peptides from a complex mixture of peptides using an immobilized modification specific antibody within the scope of the present claims. Indeed, at the time, the accepted belief in the field of peptide isolation was that post-translational modification-specific antibodies, such as phospho-specific antibodies, were *not suitable* for isolating modified peptides from complex mixtures (*see* Mann, *supra.*; Conrads, *supra.*) Further, as noted in the Background of the application (see p. 3, lines 12-30), prior art HPLC approaches have largely been unsuitable for isolation of modified peptides. Accordingly, the methods of the present invention are non-obvious and surprising over the prior art, and fulfill a long-felt but unsolved need in the art of isolating modified peptides from complex peptide mixtures (*see* Conrads, *supra.*; Mann (1999) *supra.*)

The third required prong of a *prima facie* showing of obviousness, the "all elements" prong, has also not been established in the present case, because Little and Pidgeon together fail to teach or suggest all elements of the presently claimed subject matter. Claim 12 is a dependent claim that relies on claim 2, itself a dependent claim that relies on claim 1. Taken together and incorporating all elements in the chain of dependency, claim 12 (as amended) is drawn to method for isolating a post-translationally modified peptide from a complex mixture of peptides by contacting the complex mixture of peptides with at least one immobilized (post-translational) modification-specific antibody in a chromatography column that is coupled to a mass spectrometer, isolating the modified peptide(s) specifically bound by the antibody, and characterizing the isolated peptide(s) by mass

spectrometry techniques. All elements of this preferred subject matter are not met by Little and Pidgeon, whether taken alone or together.

The limitations of Little, which discloses isolation of peptides either by introducing artificial tags or using peptide sequence-specific reagents, are discussed at length above. This reference fails to disclose or suggest the suitability of using a post-translational modification specific antibody (*i.e.* one that specifically binds a single modified amino acid or a recurring motif comprising it) to selectively isolate, from complex mixture of peptides, a population of modified peptides containing a modified residue or motif of interest. As noted, the unsuitably of prior art approaches – such as the Little methodology – existing at the time the present application was expressly noted in the Mann *et al.* review (discussed above). The inability of such approaches to selectively isolate modified peptides present in low concentrations in complex mixtures was further noted in Mann et al., Nature Biotech. 17: 954-955 (1999) (Ref. CK), which called the identification of post-translationally modified peptides a "largely unsolved problem" at the time. Pidgeon, as discussed above, relates solely to the isolation of chemical compounds from mixtures of such compounds, and fails in any way to teach, suggest, or motivate the selective isolation of modified peptides using post-translational modification specific antibodies. Thus, the limitations of Little are not cured by Pidgeon, and these references combined fail to teach, suggest, or make obvious the subject matter of claim 12.

Applicants submit that *a prima facie* showing of obviousness has not been established, since none of the three required elements of such a showing have been met. The subject matter of claim 12 (and the other pending claims) is non-obvious and patentable over Little and Pidgeon, and Applicants respectfully request that the present rejection be withdrawn. MPEP §§2142, 2143.01.

**(II)** The Examiner has also rejected claims 13-16, 30, 32 and 34 under 35 U.S.C. §103(a) as allegedly being obvious given Little (U.S. Pat. No. 6,322,970; *see supra.*) in view of Goshe (U.S. Pat. No. 6,818,970, "Phosphoprotein Binding Agents and Methods of Their Use," (issued November 16, 2004) (hereinafter "Goshe")). The Examiner acknowledges that Little fails to teach the selective isolation of phosphopeptides using an immobilized motif-specific, context-independent antibody specific for a motif comprising at least one phosphorylated amino acid. Nonetheless, the Examiner alleges that Goshe teaches the fractionation of phosphopeptides by reversed phase liquid chromatography (LC), and that the combination of this approach with the method of Little renders the presently claimed subject matter obvious. Applicants respectfully disagree, and submit that the Examiner has failed to establish a *prima facie* showing that the presently claimed method is obvious in view of the cited references.

As noted above an Examiner must establish *a prima facie* case of obviousness by establishing three elements. *See* MPEP §§2142, 2143. The combination of Little and Goshe meet none of these

three required elements, hence a *prima facie* case has not been established.

Turning to the first prong, the mere fact that references *can* be combined is *not* sufficient to establish desirability or motivation to do so (*see* MPEP §2143.01, citing *In re Mills*, 916 F.2d 680) (Fed. Cir. 1990)). Presently, the Examiner has essentially argued that the teachings of Little and Goshe *can* be combined and allegedly render the present invention obvious, but has failed to establish any evidence of a suggestion or motivation in the references themselves or in the field to specifically do so. Indeed, the cited references are each concerned with distinctly different technology. Little relates to a method for isolating a desired peptide using an immobilized peptide-specific capture reagent and then determining its identity by MS. In contrast, Goshe is directed to a method for characterizing phosphorylated protein in a sample by replacing naturally-occurring phosphate groups on proteins with isotopically-labeled organic tags that differentially label particular proteins, then isolating proteins bearing particular tags with tag-specific capture molecules (*e.g.* biotin, avidin) (*see* Goshe, *e.g.* at column 2, line 37 to column 3, line 45). There is simply no suggestion provided in Goshe to combine the isotopic labeling approach it discloses with the peptide sequence-specific or artificial tag (*e.g.* His-tag)-specific isolation method disclosed in Little, or vice versa. One of skill in the art of peptide isolation following the method taught by Little would in no way be motivated to combine it with the approach of Goshe, since the latter teaches a completely distinct approach to isolating proteins that have been artificially tagged with specific organic linkers in place of the naturally occurring phosphates. Indeed, even if such artisan were to so combine the teachings, he/she would not arrive *at the presently claimed subject matter*, which utilizes post-translational modification-specific antibodies. Accordingly, the first required prong of a *prima facie* showing of obviousness has not been met, and the rejections should be withdrawn on this ground alone.

The second required prong of a *prima facie* showing of obvious has also not been established because, even if an artisan were, *arguendo*, to have a reasonable expectation of success in combining the teachings of Little and Goshe, the expectation of success would only extend to performing the peptide isolation method disclosed in Little and incorporating the phosphate replacement/isotopic labeling approach disclosed in Goshe. Such artisan would have no expectation of success of being able to effectively and directly immunoaffinity isolate a desired population of post-translationally modified peptides from a complex mixture of peptides *using an immobilized post-translational modification specific antibody* within the scope of the present claims. In fact, Goshe itself teaches away from the present invention by requiring the *replacement* of protein phosphate groups with an artificial organic linker-plus-tag. Thus, a phosphorylation-specific antibody is not even suitable in the practice of the Goshe method. Furthermore, at the time the instant application was filed, the accepted belief in the field of peptide isolation was that post-translational modification-specific antibodies, such as phospho-specific antibodies, were *not suitable* for isolating post-translationally

modified peptides from complex mixtures (*see* Mann, *supra.*; *Conrads, supra.*) Accordingly, the second required prong of a *prima facie* showing has not been met, and the rejections should be withdrawn.

The third required prong of a *prima facie* showing of obviousness, the "all elements" prong, has also not been established in the present case, because Little and Goshe together fail to teach or suggest all elements of the presently claimed subject matter. Claim 13-16 are dependent claims that rely on claim on claim 1. Claim 1 itself (as amended) is drawn to method for isolating a post-translationally modified peptide from a complex mixture of peptides by contacting the complex mixture of peptides with at least one immobilized (post-translational) modification-specific antibody, and isolating the modified peptide(s) specifically bound by the antibody. Little alone fails to teach all elements of this method, as discussed at length earlier. Claims 13-16 further require that the post-translationally modified peptides isolated are phosphopeptides, and further that the peptides are isolated using a motif-specific, context-independent antibody that specifically binds either a single phospho-amino acid or a motif containing it. Claims 30 and 32 require the same elements as dependent claims 13-16, and further require the characterization of the isolated phosphopeptide by MS techniques. As acknowledged by the Examiner, Little alone fails to teach all of these limitations.

Goshe fails to teach the elements missing from Little. Goshe, as discussed above, relates solely to the isolation of proteins that are artificially and differentially labeled with organic linker-plus-tag isotopic labels in place of phosphate groups, using a tag-specific capture approach. Goshe fails in any way to teach, suggest, or motivate the selective and direct isolation of phosphopeptides using immobilized phosphorylation-specific antibodies. Indeed, such antibodies would not be suitable for the Goshe approach since naturally occurring protein phosphate groups are removed and replaced with the isotopic labels required in the method. The exemplary peptide isolation disclosed in Goshe and cited by the Examiner (*see* column 16, lines 10-50) in fact isolated peptides that had been artificially labeled with a biotin tag-plus-linker using the well-known ImmunoPure® avidin-biotin system. This type of tag-based approach is not within the scope of the present invention. Complicated manipulation and modification of target peptides prior to actual isolation distinguish the method disclosed in Goshe (and Little) from the present invention, which does not require them. The advantages of the instant invention -- such as the ability to *directly* isolated phosphopeptides from complex mixtures using immobilized phosphoresidue- or phospho motif-specific antibodies – over prior art approaches, such as Little, are discussed at length in the Specification (*see* pgs. 20-21). The limitations of Little are not cured by Goshe, and these references combined fail to teach, suggest, or make obvious the subject matter of claims 13-16, 30 and 32.

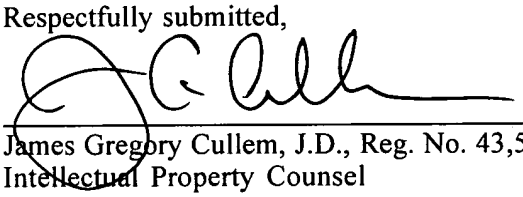
Accordingly, Applicants submit that *a prima facie* showing of obviousness has not been established, as none of the three required elements have been established. The subject matter of

claims 13-16, 30, and 32 (and the other pending claims) is non-obvious and patentable over Little and Pidgeon, and Applicants respectfully request that the present rejection be withdrawn. MPEP §§2142, 2143.01. Indeed, the methods of the present invention are a surprising advance over the prior art, and fulfill a long-felt but unsolved need in the art of modified peptide isolation from complex peptide mixtures (*see* Mann, *supra.*; Conrads, *supra.*)

**Conclusion**

The present claims are patentable over the prior art, and believed to be in condition for immediate allowance. Reconsideration and withdrawal of the outstanding objections and rejections is respectfully requested, and early and favorable allowance of these claims is earnestly solicited. If there are any questions regarding these amendments and remarks, the Examiner is requested to call the undersigned attorney at the telephone number provided.

Respectfully submitted,



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